Communication

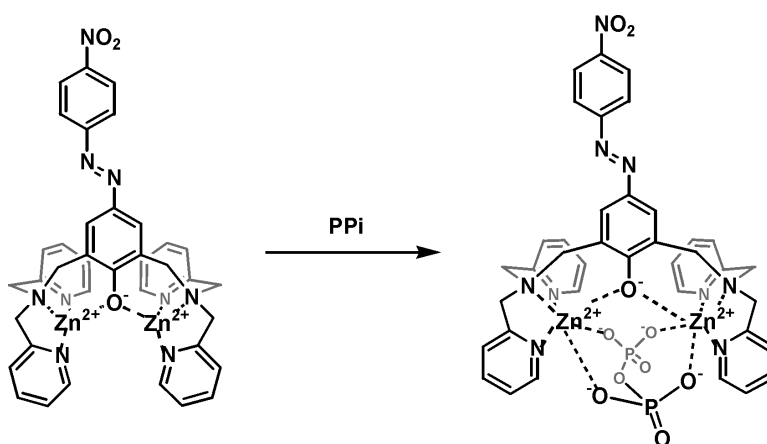# An Azophenol-based Chromogenic Pyrophosphate Sensor in Water

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An Azophenol-based Chromogenic Pyrophosphate Sensor in Water

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The development of receptors and sensors for biologically important anions is emerging as a research area of great importance.[1] Pyrophosphate anion (PPi), in particular, participates in several bioenergetic and metabolic processes.[2,3] Therefore, the detection of PPi has been the main focus of several research groups. While PPi analysis such as ion chromatography remains important, there is mounting incentive to find alternative means of analysis, including those based on the use of selective chemosensors.[1b,4] Particularly useful would be systems that can recognize PPi in an aqueous solution and signal its presence via an optical signal. Until now, very few examples of optical sensors for PPi in aqueous solution have been reported.[5,6] In this communication, we present a new chromogenic PPi sensor based on an azophenol-Dpa (bis(2-pyridylmethyl)amine) system, which shows a high sensitivity and selectivity for PPi over other anions in aqueous solvent of a wide pH range.[7]

A color-inducing *p*-nitrophenylazo group can be easily introduced on the para position of the phenolic moiety of 2,6-bis[(bis(2-pyridylmethyl)amino)methyl]phenol (H-bpp).[8] Zn^{2+} complexation with bis(2-pyridylmethyl)amine (Dpa) moiety generates an anion-binding site through the formation of a well-known phenoxo-bridged dinuclear metal complex (Scheme 1).[9] Compound **1** was obtained in an overall yield of 38% from 2,6-dimethylphenol.[10a] Sensor **1·2Zn**, the dinuclear Zn^{2+} complex of compound **1**, is easily formed by the addition of a methanolic solution of **1** to an aqueous solution of 2 equiv of $Zn(NO_3)_2$.

First, the effect of anions (sodium salts) on the absorption spectrum of **1·2Zn** (30 μM) was examined in an aqueous solution of 10 mM HEPES buffer (pH 7.4) (HEPES = 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid) at 25 °C (Figure 1). In the absence of an anion guest, the absorption spectrum of sensor **1·2Zn** is characterized by an intense band centered at 417 nm.

Sensor **1·2Zn** does not show any obvious spectral change upon addition of $H_2PO_4^-$ as well as other monovalent anions such as $CH_3CO_2^-$, F^-, HCO_3^-, Cl^- even up to an excess of 100 equiv. Moreover, no detectable spectral change is observed upon addition of dibasic anions HPO_4^{2-} and citrate. However, the addition of $P_2O_7^{4-}$ (PPi) causes bathochromic shifts from 417 nm (λ_{max}) to 465 nm. It is remarkable that the degree of absorption changes is no longer affected by the addition of more than 1 equiv of PPi. As expected from the UV−vis absorption data, color change occurs by the addition of PPi to the solution of **1·2Zn** from yellow to red. Job's plot for the binding between **1·2Zn** and PPi shows a 1:1 stoichiometry (inset of Figure 1a).[11] Even in the presence of 10 equiv of HPO_4^{2-}, sensor **1·2Zn** shows a similar detection ability for PPi. It is surprising that the apparent association constant (K_a) was determined as $(6.6 \pm 1.2) \times 10^8$ M^{-1} for PPi−**1·2Zn** by a standard algorithm for competitive binding in the presence of excess HPO_4^{2-} in a pure aqueous solvent of 10 mM HEPES buffer (pH

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Scheme 1. Synthetic Strategy for Sensor **1·2Zn**[a]
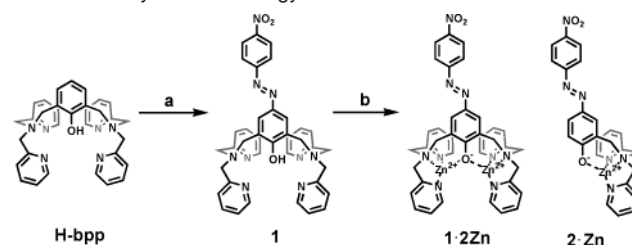
[a] Conditions: (a) i. aq. NaOH, MeOH, rt, ii. *p*-nitroaniline, NaNO₂, aq. HCl, acetone, 0 °C. (b) $Zn(NO_3)_2$, H_2O, MeOH.

7.4) at 25 °C.[11] These results suggest that sensor **1·2Zn** has high selectivity for PPi over other anions.

Similar results are obtained in an aqueous solvent of 100 mM HEPES buffer (pH 7.4) at 25 °C. The addition of PPi also makes a color change from yellow ($\lambda_{max} = 417$ nm) to red ($\lambda_{max} = 463$ nm). In 100 mM HEPES buffer, sensor **1·2Zn** shows the reduced affinity for PPi ($K_a = (8.3 \pm 1.8) \times 10^7$ M^{-1}), compared with 10 mM HEPES buffer system.

The novel binding mode for PPi−**1·2Zn** is illustrated in Figure 2, which was unambiguously elucidated by an X-ray analysis. The X-ray structure of the complex reveals that the two sets of oxygen anions on each P of PPi bind to the dinuclear zinc complex by bridging the two metal ions to give rise to the two hexacoordinated Zn^{2+} ions in **1·2Zn**.[12a] The binding mode for HPO_4^{2-}−**1·2Zn** should be the same as that of HPO_4^{2-}-H-bpp.[9c−f] Despite this, HPO_4^{2-} does not make a large UV-vis absorption change upon complexation with **1·2Zn**. Instead, only PPi induces the selective red-shift of λ_{max} of **1·2Zn** because weakening the bond between *p*-nitrophenylazo phenolate oxygen and Zn^{2+} induces more negative charge character on the phenolate oxygen and thus the bathochromic shift of λ_{max} of **1·2Zn** occurs. As revealed by previous works,[9c−f] HPO_4^{2-} does not coordinate in tetradentate fashion as PPi does. This explains why HPO_4^{2-} does not alter λ_{max} of **1·2Zn**. Stronger coordination of PPi to dinuclear zinc complex enables sensor **1·2Zn** to show color changes and higher selectivity over HPO_4^{2-} (Figure 3). Hexacoordination of Zn^{2+} ions is clearly reflected in the extremely high K_a of PPi−**1·2Zn** in water ($K_a = 6.6 \times 10^8$ M^{-1}). It is worthwhile noting that PPi binds **1·2Zn** over 10^3-fold more tightly than HPO_4^{2-} does.[9e]

A control sensor, mononuclear **2·Zn** does not show λ_{max} and color changes upon the addtion of PPi. This result means that the cooperative action of two Zn^{2+}−Dpa is needed for the selective sensing of PPi.[5d] Finally, to check the working pH, the effect of the pH value of the medium on the PPi sensing was checked. UV−vis absorption changes shown in Figure 1b occur in the wide pH range of 6.5−8.3 with a similar tendency.[10b] This result shows that even if the external pH is disturbed, sensor **1·2Zn** can still detect PPi.

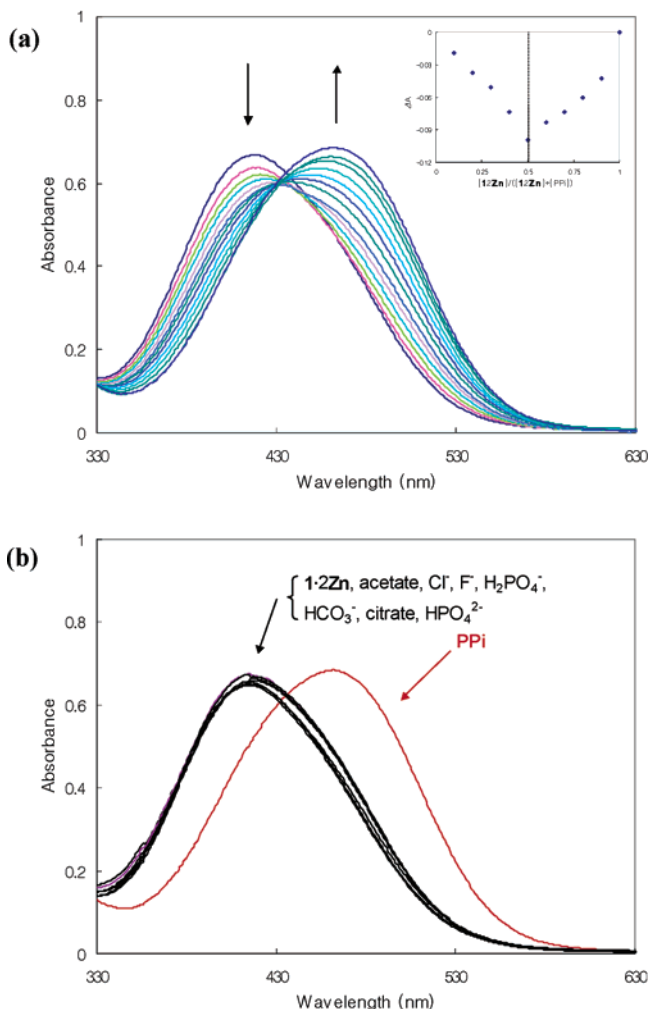In summary, we have developed a new azophenol-based colorimetric sensor, which shows a selective coloration for PPi with

Figure 1. (a) Absorbance change of sensor **1·2Zn** (30 μM) upon addition of PPi (Sodium salt): [PPi] = 0, 2, 4, 6, 8, 11, 14, 17, 20, 23, 26, 29, 32 μM. The spectra were measured in an aqueous solvent of 10 mM HEPES buffer (pH 7.4) at 25 °C. (Inset) The Job's plot examined between **1·2Zn** and PPi. (b) UV−vis spectra of sensor **1·2Zn** (30 μM) in an aqueous solvent 10 mM HEPES buffer (pH 7.4) at 25 °C in the presence of various anions (30 μM).
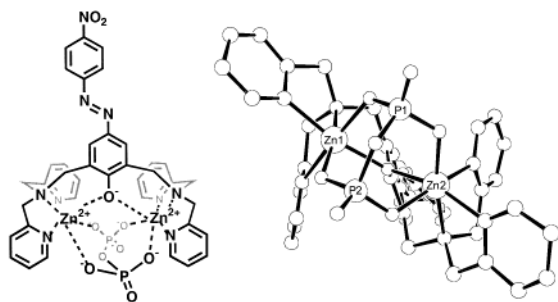

Figure 2. (Left) Binding mode. (Right) PPi−**1·2Zn** with the partial atom labeling scheme (30% probability level).[12]

high affinity in aqueous solution in a wide pH range.[13] This system shows good selectivity for PPi even in the presence of a strong competitor such as HPO_4^{2-}. In addition, the novel binding mode for PPi−**1·2Zn** was unambiguously confirmed by an X-ray analysis. For biochemical and analytical applications, work is directed toward the development of fluorescent sensors capable of detecting PPi at lower concentrations.

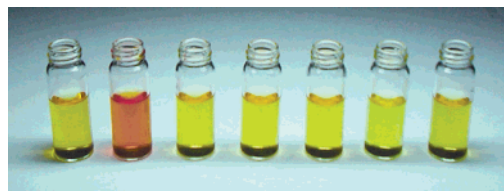**Figure 3.** Color changes of sensor **1·2Zn** in 10 mM aqueous HEPES buffer solution (pH 7.4), [**1·2Zn**] = 60 μM, [anion] = 60 μM; from left to right: no anion, PPi, citrate, HPO_4^{2-}, $H_2PO_4^{-}$, acetate, F^{-}.

Supporting Information Available: Experimental procedure and selected spectral data for compounds **1**, **2**, **1·2Zn**, and **2·Zn**, UV−vis absorption data (PDF). X-ray crystallography data (CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(7) To the best of our knowledge, our system is the first colorimetric sensor for PPi in water that holds several advantages when compared to previous systems for the following reasons. Our system does not have any competing simple inorganic anions, as measured by UV−vis studies, unlike the Kikuchi system[5d] which also responds to citrate anions. Furthermore, our system operates in a wide pH range, unlike the previous systems.[5a,d]

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(10) See the Supporting Information for: (a) detailed syntheses (b) pH dependence of sensor **1·2Zn**.

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(12) (a) Although refinement of the structure has been unsatisfactory (R1 = 0.14) due to the disorder of solvents and cation molecule, it clearly shows the connectivity of the complex that we are interested in. (b) Some atoms are omitted for clarity

(13) Naked eye detection for PPi is optimized under the condition of [**1·2Zn**] = 60 μM.

JA034689U